

Successful Hematopoietic Stem Cell Transplantation in 2 Children with X-Linked Chronic Granulomatous Disease from Their Unaffected HLA-Identical Siblings Selected Using Preimplantation Genetic Diagnosis Combined with HLA Typing

Evgenios Goussetis,¹ Christopher P. Konialis,² Ioulia Peristeri,¹ Vasiliki Kitra,¹ Maria Dimopoulou,¹ Theoni Petropoulou,³ George Vessalas,¹ Andreas Papassavas,⁴ Marianna Tzanoudaki,⁵ Georgia Kokkali,⁶ Eftychia Petrakou,¹ Antonia Spiropoulos,¹ Constantinos G. Pangalos,² Konstantinos Pantos,⁶ Stelios Graphakos¹

We report 2 children with X-linked chronic granulomatous disease (X-CGD) who underwent hematopoietic stem cell transplantation (HSCT) using grafts from their siblings selected before implantation to be both unaffected and HLA-matched donors. Preimplantation genetic diagnosis (PGD) along with HLA-typing were performed on preimplantation embryos by single-cell multiplex polymerase chain reaction using informative short tandem repeat markers in the HLA locus together with the gene region containing the mutations. Two singleton pregnancies resulted from the intrauterine transfer of selected embryos; these developed to term, producing 1 healthy female and 1 X-CGD carrier female, which are HLA-identical siblings to the 2 affected children. Combined grafts of umbilical cord blood (UCB) and bone marrow (BM) stem cells were administered to the recipients after myeloablative (MA) conditioning at the ages of 4.5 years and 4 years, respectively. Both patients are well, with complete donor hematopoietic and immunologic reconstitution, at 18 and 13 months posttransplantation, respectively. This report demonstrates that HSCT with HLA-matched sibling donors created by PGD/HLA typing of in vitro fertilized embryos is a realistic therapeutic option and should be presented as such to families with children who require a non-urgent HSCT but lack an HLA-genotypical donor.

Biol Blood Marrow Transplant 16: 344-349 (2010) © 2010 American Society for Blood and Marrow Transplantation

KEY WORDS: Preimplantation genetic diagnosis/HLA testing, Hematopoietic stem cell transplantation, X-linked chronic granulomatous disease

From the ¹Stem Cell Transplant Unit, Aghia Sofia Children's Hospital, Athens, Greece; ²Department of Molecular Genetics and Preimplantation Genetic Diagnosis, Intergenetics Hellas, Diagnostic Genetic Center, Athens, Greece; ³First Pediatric Clinic, Aghia Sofia Children's Hospital Medical School, University of Athens, Athens, Greece; ⁴Hellenic Cord Blood Bank, Foundation for Biomedical Research, Academy of Athens, Athens, Greece; ⁵Immunology Department, Aghia Sofia Children's Hospital, Athens, Greece; and ⁶Center for Human Reproduction, Genesis Athens Hospital, Athens, Greece.

Financial disclosure: See Acknowledgments on page 348.

Correspondence and reprint requests: Evgenios Goussetis, MD, SCT-Unit, Aghia Sofia Children's Hospital, Thivon and Mikras Asias, 11527 Athens, Greece (e-mail: evgoussetis@gmail.com).

Received September 3, 2009; accepted October 10, 2009

© 2010 American Society for Blood and Marrow Transplantation

1083-8791/10/163-0006\$36.00/0

doi:10.1016/j.bbmt.2009.10.010

INTRODUCTION

X-linked chronic granulomatous disease (X-CGD) is an inherited defect in phagocyte nicotinamide adenine dinucleotide phosphate oxidase resulting from a deficiency of gp91^{phox} component. At the molecular level, X-CGD is caused by mutations affecting the *CYBB* gene on chromosome Xp21.1 [1,2]. As a result of impaired phagocytic ability, affected persons suffer from severe recurrent infections, both bacterial and fungal, resulting in the formation of infiltrating granulomatous lesions. Despite prophylactic therapy, long-term survival remains poor; only 50% of patients are alive at the age of 30 years [3,4]. Allogeneic hematopoietic stem cell transplantation (HSCT) using an HLA-matched

sibling donor (MSD) has achieved long-term cure of X-CGD in 80% to 90% of cases [5,6]. A healthy MSD is not available in the vast majority of the cases, however. For such patients, a matched unrelated donor (MUD) is a suitable option, but at the cost of increased treatment-related morbidity and mortality (TRM) [5,7]. Preimplantation genetic diagnosis (PGD) for single-gene diseases was developed to help families desiring a new pregnancy to select unaffected embryos in vitro, thereby avoiding the need for pregnancy termination. Recently, this strategy has been combined with HLA typing with the aim of selecting suitable histocompatible embryos as potential stem cell donors for affected siblings [8-10]. There is limited experience with this methodology and even less with its completed clinical application, however.

METHODS

Patient Characteristics

Patient 1

In this 4.5-year-old boy with an established diagnosis of X-CGD since infancy, diagnosis was confirmed by respiratory burst screening tests and by DNA *CYBB* gene sequencing (R73X C → T mutation). Despite both antimicrobial and interferon- γ prophylaxis, the patient had recurrent infections from the age of 4 months onward, and granulomatous colitis was diagnosed at the age of 1.7 years.

Patient 2

This 4-year-old boy was diagnosed with X-CGD at age 6 months by both functional and molecular tests (R157X C → T mutation). Before presenting for HSCT, he had experienced multiple cervical abscesses despite receiving antibiotic prophylaxis.

Donor Selection

Neither patient had an HLA-genoidentical sibling donor. The patients' parents came to the Diagnostic Genetic Centre in Athens on their own initiative for genetic consultation. After detailed genetic counseling, the couples came to the Centre for Human Reproduction, Genesis Athens Hospital for complete counseling regarding in vitro fertilization (IVF) and embryo biopsy procedures. The clinical protocol was approved by the center's Institutional Research and Ethics Committee for the IVF treatment, embryo biopsy, and PGD procedures required for the transfer of unaffected and HLA-identical embryos to the mother's uterus. Written informed consent concerning the PGD/HLA typing procedure, as well as the cryopreservation of nontransferred embryos for future use, in accordance with the Helsinki Declaration, was

obtained from both couples. A single cycle of IVF was initiated for each couple, and standard hormonal stimulation and culture techniques were used to generate embryos for PGD and HLA genotyping [11,12]. All fertilized embryos were cultured and biopsied by laser on day 3 in the first couple and on day 5 for the second couple [13]. Embryo genotyping was performed using a nested multiplex polymerase chain reaction (PCR) approach, involving first- and second-round amplification of the informative polymorphic short tandem repeat (STR) markers previously selected by extensive HLA genotyping and haplotype analysis of the family members. Detection of the disease mutation was performed by minisequencing [14]. Embryos identified to be HLA-identical to the patients and unaffected by X-CGD (homozygous wild-type allele or heterozygous mutation) were transferred 6 days after fertilization to the uterus of the respective mothers. The 2 singleton full-term pregnancies resulted in the birth of 1 healthy and 1 X-CGD carrier female siblings. Confirmatory genetic testing was performed using chorionic villus sampling in the first trimester for CGD mutations and HLA typing. To rule out the possibility of extreme inactivation of the X chromosome in the case of the heterozygotic sibling donor carrying the nonmutated *CYBB*, postnatal neutrophil superoxide production was measured.

HSCT

Umbilical cord blood (UCB) was collected, frozen, and stored in liquid nitrogen as described previously [15]. Given the limited experience with UCB transplantation (UCBT) in patients with X-CGD, we decided to combine UCB and bone marrow (BM) HSC from the same donors, to ensure stable long-term engraftment. Therefore, after parental written consent was provided, when the 2 sibling donors reached age 16 months, reduced-volume BM was harvested. Both patients were conditioned with busulfan (Bu; 16 mg/kg i.v) and cyclophosphamide (Cy; 200 mg/kg i.v.). Graft-versus-host disease (GVHD) prophylaxis comprised cyclosporine A and a short course of methotrexate (MTX). Time to white blood cell engraftment was defined as the first of 3 consecutive days with a neutrophil count $> 0.5 \times 10^9/L$, and platelet engraftment was defined as the first of 3 consecutive days on which a platelet count $> 20 \times 10^9/L$ was maintained without transfusion. To assess engraftment and the degree of chimerism, patients were monitored at different time points, using a PCR assay to identify genetic polymorphism of STRs. Superoxide production by neutrophils was measured by the dihydrorhodamine (DHR) test. Expression of gp91^{phox} on neutrophils was measured using the 7D5 monoclonal antibody (mAb) and flow cytometry.

RESULTS

PGD/HLA Typing of Embryos

Each family was subjected to a single clinical IVF cycle. A total of 48 embryos were tested for the 2 couples. Eight of these embryos were diagnosed as unaffected and HLA-matched, and 2 embryos from each cycle were transferred, resulting in 2 singleton pregnancies and 2 newborns, 1 healthy and 1 X-CGD carrier. All X-CGD-unaffected embryos not transferred were cryopreserved for eventual future use. Table 1 summarizes the PGD/HLA typing results.

Graft Characteristics

The numbers of UCB-nucleated and CD34⁺ cells collected from the first donor ($1.4 \times 10^7/\text{kg}$ and $3.5 \times 10^4/\text{kg}$, respectively) were deemed insufficient to secure long-term engraftment. Therefore, we decided to increase the number of hematopoietic progenitors by harvesting a reduced BM volume from the same donor. In the case of second donor, and despite an adequate number of cells in the UCB unit, we decided to harvest a small volume (100 mL) of BM to further enrich the number of hematopoietic progenitors. Co-infusion of BM cells in the second case was chosen because of the extremely limited experience with matched related UCBT in X-CGD and the high risk of graft failure reported in small series of children undergoing unrelated UCBT [16]. In both cases, BM was harvested when the donors reached the age of 16 months. Details on the number of nucleated and progenitor cells of the grafts are given in Table 2.

Transplantation Outcome

Both patients engrafted quickly after receiving a combined graft of UCB and BM cells. Neutrophil engraftment was achieved on day +20 in patient 1 and on day +18 in patient 2, and platelet engraftment occurred on days +19 and +17, respectively. Complete donor-derived hematopoietic chimerism was repeatedly detected after day +60 posttransplantation by studying STRs. Neither patient developed acute or chronic graft-versus-host disease (aGVHD, cGVHD). Cyclosporine (CsA) and replacement i.v. immunoglobulin were stopped at 6 months posttransplantation. Patient 1 had normal stools since day +30 posttransplantation, indicating resolution of colitis. In patient 1, both DHR and gp91^{phox} expression were restored to normal on 100% of the neutrophils posttransplantation, whereas in patient 2, only 50% of neutrophils exhibited normal DHR and gp91^{phox} expression posttransplantation. Repeated testing revealed similar results at various time intervals posttransplantation (Figure 1). At their last follow-up visit, both patients were in excellent clinical condition, demonstrating normal hematopoietic and immunologic reconstitu-

Table 1. Summary of the PGD/HLA typing results

Family	IVF Cycle	Embryos Formed	HLA-Matched, CGD-Unaffected Embryos	Embryos Transferred	Pregnancies
1	1	37	6*	2	1
2	1	11	2†	2	1

PGD indicates preimplantation genetic disease; CGD, chronic granulomatous disease. Biopsies were performed at the cleavage stage (day 3 after fertilization) for family 1 and at the blastocyst stage (day 5 after fertilization) for family 2.

*Four embryos were homozygous for the wild-type allele.

†Both embryos were carriers for the CGD mutation.

tion at 18 and 13 months posttransplantation, respectively.

DISCUSSION

The practice of conceiving an additional child with the intention of using it as an HSC donor for an affected sibling has been done for many years [17]. In the early years, parents planned to have another child by natural conception and hoped for an HLA-matching sibling. More recently, Verlinsky et al. [18] reported HLA typing combined with PGD in a case of Fanconi anemia. Although only 3/16 embryos for X-linked recessive inheritance will be both disease-free and HLA-identical, the likelihood of conceiving a matched donor using PGD/HLA typing is much greater than that with a spontaneous conception. Only a limited number of PGD centers offering HLA typing on human preimplantation embryos exist worldwide, limiting experience with the combination of IVF and PGD to achieve pregnancy and birth of a healthy HLA-compatible sibling. Published data from different series worldwide have reported 64 children born after PGD/HLA testing; the pregnancy rate per embryo transfer was 34% on average, varying depending on the IVF center and age of the mother [10,19,20]. All 64 children were healthy and HLA-identical to their affected

Table 2. Characteristics of patients, donors, and grafts, and transplantation outcome

	Patient 1	Patient 2
Age, years	4.5	4
Sex	Male	Male
Donor sex	Female	Female
X-CGD carrier donor	No	Yes
UCB volume collected, mL	112	143
UCB nucleated cells/kg	1.4×10^7	10×10^7
UCB CD34 ⁺ cells/kg	0.35×10^5	2.8×10^5
BM volume collected, mL	174	100
BM nucleated cells/kg	3.2×10^8	3×10^8
BM CD34 ⁺ cells/kg	13.6×10^6	7.5×10^6
Neutrophil engraftment	Day +20	Day +18
Platelet engraftment	Day +19	Day +17
GVHD	No	No
Chimerism	Full donor	Full donor

CGD indicates chronic granulomatous disease; UCB, umbilical cord blood; BM, bone marrow; GVHD, graft-versus-host disease.

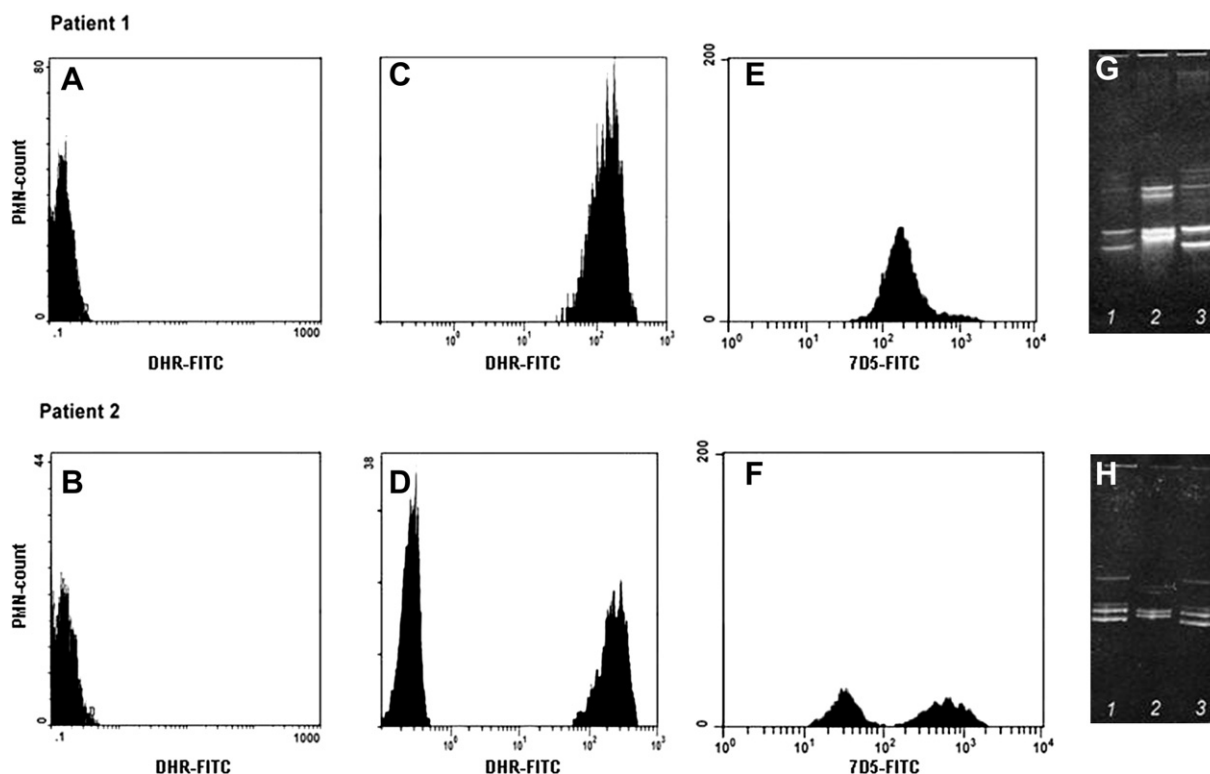


Figure 1. Oxidative burst activity of neutrophils, expression of gp91^{phox}, and hematopoietic chimerism. Both H₂O₂-producing granulocytes after phorbol myristate acetate stimulation by fluorescent activated cell sorting analysis (DHR test) and expression of gp91^{phox} posttransplantation were corrected after transplantation. DHR before transplantation (A and B) showed neutrophils with no oxidative burst activity, whereas both DHR (C and D) and gp91^{phox} expression (E and F) at 18 and 13 months posttransplantation, respectively, for patients 1 and 2 showed 100% corrected neutrophils for patient 1 and a 50% mosaicism for patient 2. Hematopoietic chimerism analysis by STR-PCR at 18 and 13 months posttransplantation, respectively, for patients 1 and 2 revealed 100% donor genotype (G and H). Lane 1, donor; lane 2, recipient; lane 3, recipient posttransplantant.

siblings. In these series, only 12 transplantations leading to the cure of affected siblings have been reported [19,21,22]. Although no mistakes on HLA typing have been reported so far, the possibility of misdiagnosis of either HLA typing or PGD resulting from technical and human errors cannot be excluded [19,23]. Therefore, it must be made clear to the family that prenatal diagnosis is an absolute requirement to rule out an affected pregnancy. In addition, the use of this method raises a number of practical and ethical issues, such as the creation of human beings solely for instrumental use, most of which can be addressed by careful genetic and psychological counseling of the parents [24-26]. Other significant open issues remain, including the fate of unused healthy HLA-nonidentical embryos that greatly exceed the number created to address infertility. The law on medically assisted reproduction should provide regulations regarding the use of PGD/HLA typing and determine the fate of unused healthy embryos. In some countries, regulations permit the transfer of selected embryos based on HLA typing in combination with PGD, whereas in others this is not allowed. Clearly, further research is required to elucidate the clinical, social, psychological, and economic issues that might be relevant to the application of this technology.

Although infusion of autologous HSC genetically modified to express gp91^{phox} has been associated with some transitory benefits in a few patients, the safety and efficacy of gene therapy in X-CGD remain of major concern [27,28]. Currently, allogeneic HSCT from an HLA-matched donor is the only therapeutic modality with curative potential for patients with X-CGD. Allogeneic HSCT is best performed at an early age in children with recurrent serious infections despite antimicrobial prophylaxis [6,29]. A recent study of the efficacy of allogeneic HSCT in 20 patients with X-CGD found that the type of donor (MSD or MUD) had no effect on transplantation outcome [30]. It is obvious, however, that the efficacy and safety of MUD-HSCT should be examined in larger cohorts of patients. Moreover, a MUD is available for only 60% to 70% of the patients in need of a donor, possibly even lower in patients of ethnic minorities [31]. Experience with either related or unrelated UCB as the source of HSC for patients with X-CGD is very limited, and only a few cases have been reported so far [32].

Following the recently published first case report of successful HSCT of an X-linked-CGD patient using preimplantation female sex typing and HLA-typing [33], we describe 2 additional patients who underwent successful transplantation from a matched

healthy sibling preselected by combined PGD and HLA typing. We chose to use PGD rather than female gender selection to detect the disease-specific mutation, to preclude the rejection of unaffected male embryos that cannot be differentiated from those affected when using fluorescein in situ hybridization (FISH) for female sex typing.

PGD/HLA typing technology should be considered and discussed with parents in cases where no suitable MSD is available and transplantation using an unrelated donor either carries significantly higher risks than the use of a sibling donor or is not possible because of the unavailability of a suitable MUD. The foregoing methodology can be applied in patients requiring nonurgent HSCT, such as patients with a genetic disease or children who may require transplantation in the future because of progression of an underlying disease, such as acute lymphoblastic leukemia (ALL) in first complete remission (CR1) (20). In addition, the parents of the affected child still must be in reproductive age. Before making any decision, parents should be informed about the efficacy and safety of all alternative options, such as allogeneic HSCT using an MUD or an unrelated UCB unit. Parents also should be informed about the possibility of using BM or reduced-volume BM plus UCB, as in our patients reported here, especially when insufficient UCB cells are available. A high cell dose ($> 3.7 \times 10^7/\text{kg}$) is considered crucial to sustain engraftment in patients with nonmalignant diseases undergoing MRD-UCBT [34]. Although in patient 2, the UCB nucleated cells would have been sufficient to secure engraftment without the addition of BM cells, we chose to combine UCB and BM to increase the cell dose because of the limited and not encouraging experience with UCBT in patients with X-CGD patients [32]. However, BM harvest, especially from a pediatric donor, is an invasive procedure and should be chosen only after UCB collection or transplantation fails. In such cases, we recommend delaying transplantation until the donor is between the ages of 12-24 months. Within this age range, depending on the recipient's weight, a reduced BM volume not exceeding 15 mL/kg donor weight should suffice. Alternative strategies pursued to obtain a high rate of engraftment in the presence of insufficient UCB include coinfusion of third-party mesenchymal stem cells [35] or mobilized CD34⁺ cells [36]. However, the available data are insufficient to offer such a strategy as an alternative to BM cells obtained from the same donor to increase the engraftment potential of the UCB graft.

In conclusion, transplant physicians should be aware of the feasibility and efficacy of the PGD/HLA typing method as well as the indications of its use. Regardless of the final decision, an immediate search for a suitable unrelated donor at the time of establishing the indication for HSCT should never be omitted.

ACKNOWLEDGMENTS

Financial disclosure: The authors thank Dr F. Fiorentino (GENOMA, Rome, Italy) for his valuable assistance with the PGD/HLA testing of our first patient. They also thank Professor B. H. Belohradsky and Dr E. Renner (Abteilung für Antimikrobielle Therapie und Infektionsimmunologie, Kinderklinik und Poliklinik der Universität München) for assessing gp91^{phox} expression.

REFERENCES

- Heyworth PG, Cross AR, Curnutte JT. Chronic granulomatous disease. *Curr Opin Immunol*. 2003;15:578-584.
- Segal BH, Leto TL, Gallin JI, et al. Genetic, biochemical, and clinical features of chronic granulomatous disease. *Medicine (Baltimore)*. 2000;79:170-200.
- Van den Berg JM, van Koppen E, Åhlin A, et al. Chronic granulomatous disease: The European experience. *PLoS ONE*. 2009;4: e5234:1-10.
- Winkelstein JA, Marino MC, Johnston RB Jr., et al. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine (Baltimore)*. 2000;79:155-169.
- Seger RA, Güngör T, Belohradsky BH, et al. Treatment of chronic granulomatous disease with myeloablative conditioning and an unmodified hemopoietic allograft: a survey of the European experience, 1985-2000. *Blood*. 2002;100:4344-4350.
- Seger RA. Modern management of chronic granulomatous disease. *Br J Haematol*. 2008;140:255-266.
- Del Giudice I, Iori AP, Mengarelli A, et al. Allogeneic stem cell transplant from HLA-identical sibling for chronic granulomatous disease and review of the literature. *Ann Hematol*. 2003;82:189-192.
- Fiorentino F, Biricik A, Karadayi H, et al. Development and clinical application of a strategy for preimplantation genetic diagnosis of single gene disorders combined with HLA matching. *Mol Hum Reprod*. 2004;10:445-460.
- Van de Velde H, Georgiou I, De Rycke M, et al. Novel universal approach for preimplantation genetic diagnosis of β -thalassemia in combination with HLA matching of embryos. *Hum Reprod*. 2004;19:700-708.
- Kuliev A, Rechitsky S, Tur-Kaspa I, et al. Preimplantation genetics. Improving access to stem cell therapy. *Ann NY Acad Sci*. 2005;1054:223-227.
- Pantos K, Meimeth-Damianaki T, Vaxevanoglou T, et al. Prospective study of a modified gonadotropin-releasing hormone agonist long protocol in an in vitro fertilization program. *Fertil Steril*. 1994;61:709-713.
- Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*. 1992;340:17-18.
- Kokkali G, Traeger-Synodinos J, Vrettou C, et al. Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation diagnosis of β -thalassemia: a pilot study. *Hum Reprod*. 2007;22:1443-1449.
- Pangalos CG, Hagrefelt B, Kokkali G, et al. Birth of a healthy histocompatible sibling following preimplantation genetic diagnosis for chronic granulomatous disease at the blastocyst stage coupled to HLA typing. *Fetal Diagn Ther*. 2008;24:334-339.
- Rubinstein P, Dobrila L, Rosenfield RE, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci USA*. 1995;92:10119-10122.
- Parikh SH, Szabolcs P, Prasad VK, et al. Correction of chronic granulomatous disease after second unrelated donor umbilical cord blood transplantation. *Pediatr Blood Cancer*. 2007;49:982-984.

17. Kearney W, Kaplan AL. Parity for the donation of bone marrow: Ethical and policy consideration. In: Blank RH, Bonnicksen AL, editors. New York: Columbia University Press; 1993 p. 262-285.
18. Verlinsky Y, Rechitsky S, Schoolcraft W, et al. Preimplantation diagnosis for Fanconi anemia combined with HLA-matching. *JAMA*. 2001;285:3130-3133.
19. Van de Velde H, De Rycke M, De Man C, et al. The experience of two European preimplantation genetic diagnosis centres on human leukocyte antigen typing. *Hum Reprod*. 2009;24:732-740.
20. Samuel GN, Strong KA, Kerridge I, et al. Establishing the role of pre-implantation genetic diagnosis with human leukocyte antigen typing: what place do "saviour siblings" have in paediatric transplantation? *Arch Dis Child*. 2009;94:317-320.
21. Grewal SS, Kahn JP, MacMillan ML, et al. Successful hematopoietic stem cell transplantation for Fanconi anemia from unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. *Blood*. 2004;103:1147-1151.
22. Bielora B, Hughes MR, Auerbach AD, et al. Successful umbilical cord blood transplantation for Fanconi anemia using pre-implantation genetic diagnosis for HLA-matched donor. *Am J Hematol*. 2004;77:397-399.
23. Wilton L, Thornhill A, Traeger-Synodinos J, et al. The causes of misdiagnosis and adverse outcomes in PGD. *Hum Reprod*. 2009;24:1221-1228.
24. Pennings G, Scots R, Liebers I. Ethical considerations on preimplantation genetic diagnosis for HLA typing to match a future child as a donor of hematopoietic stem cells to a sibling. *Hum Reprod*. 2002;17:534-538.
25. Fost NC. Conception for donation. *JAMA*. 2004;291:2125-2126.
26. De Wert Q, Liebers I, Van de Velde H. The future (r)evolution of preimplantation genetic diagnosis/human leukocyte antigen testing: Ethical reflections. *Stem Cells*. 2007;25:2167-2172.
27. Malech HL, Maples PB, Whiting-Theobald N, et al. Prolonged production of NADPH oxidase-corrected granulocytes after gene therapy of chronic granulomatous disease. *Proc Natl Acad Sci USA*. 1997;94:12133-12138.
28. Ott MG, Schmidt M, Schwarzwaelder K, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nat Med*. 2006;12:401-409.
29. Kang EM, Malech HL. Advances in treatment for chronic granulomatous disease. *Immunol Res*. 2009;43:77-84.
30. Soncini E, Slatter MA, Jones LBKR, et al. Unrelated donor and HLA-identical sibling hematopoietic stem cell transplantation cure chronic granulomatous disease with good long-term outcome and growth. *Br J Haematol*. 2009;145:73-83.
31. Rocha V, Locatelli F. Searching for alternative hematopoietic stem cell donors for pediatric patients. *Bone Marrow Transplant*. 2008;41:207-214.
32. Mochizuki K, Kikuta A, Ito M, et al. Successful unrelated cord blood transplantation for chronic granulomatous disease: a case report and review of the literature. *Ped Transplant*. 2009;13:384-389.
33. Reichenbach J, Van de Velde H, De Rycke M, et al. First successful bone marrow transplantation for X-linked chronic granulomatous disease by using preimplantation female gender typing and HLA matching. *Pediatrics*. 2008;122:e778-e782.
34. Rocha V, Chastang C, Souillet G, et al. Related cord blood transplants: the Eurocord experience from 78 transplants. Eurocord Transplant group. *Bone Marrow Transplant*. 1998;21(Suppl 3):S59-S62.
35. Macmillan ML, Blazar BR, DeFor TE, et al. Transplantation of ex-vivo culture-expanded parental haploidentical mesenchymal stem cells to promote engraftment in pediatric recipients of unrelated donor umbilical cord blood: results of a phase I-II clinical trial. *Bone Marrow Transplant*. 2009;43:447-454.
36. Bautista G, Cabrera JR, Regidor C, et al. Cord blood transplants supported by co-infusion of mobilized hematopoietic stem cells from a third-party donor. *Bone Marrow Transplant*. 2009;43:365-373.